Multifaceted involvement of microglia in gray matter pathology in multiple sclerosis

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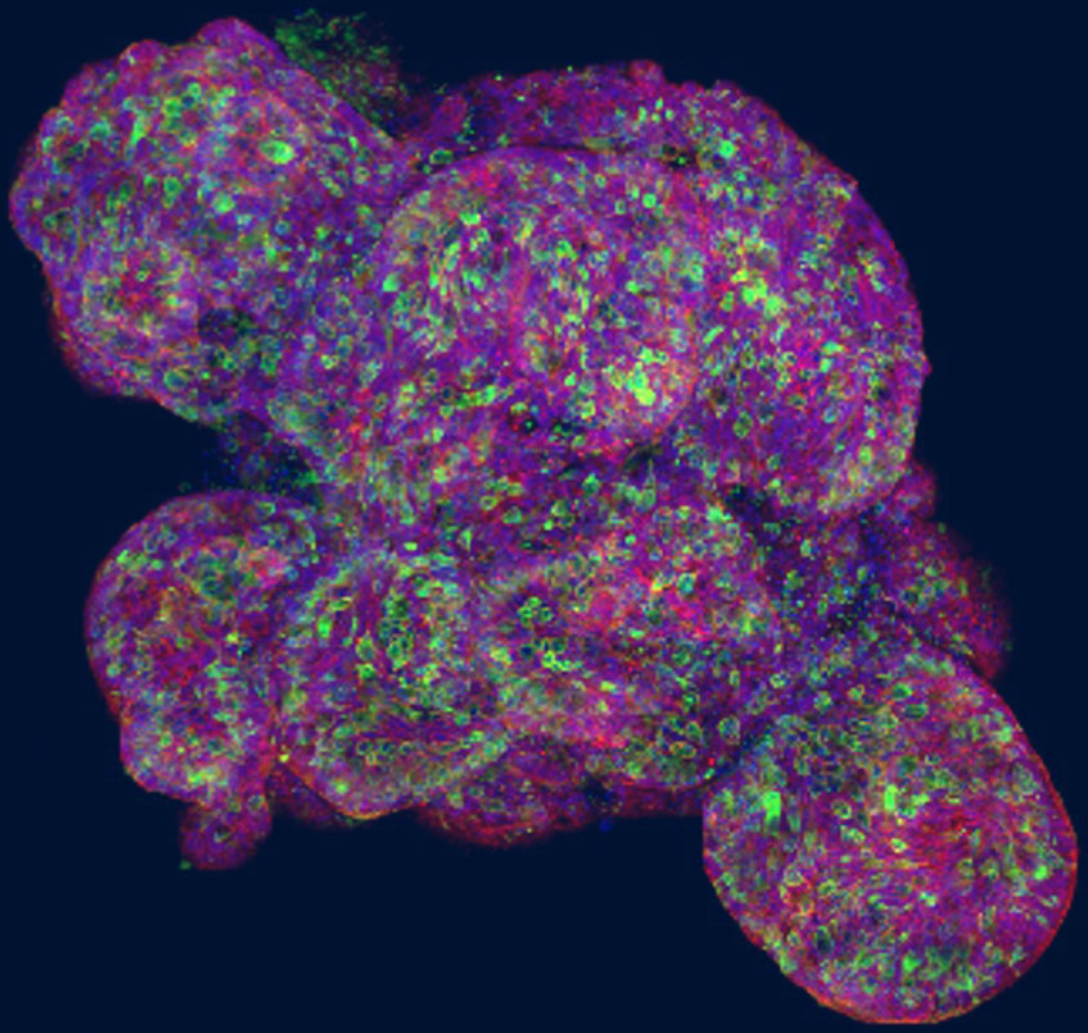

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Multifaceted involvement of microglia in gray matter pathology in multiple sclerosis

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Abstract

In the inflammatory demyelinating neurodegenerative disease multiple sclerosis (MS), there is increasing interest in gray matter pathology, as neuronal loss and cortical atrophy correlate with disability and disease progression, and MS therapeutics fail to significantly slow or stop neurodegeneration. Microglia, the central nervous system (CNS)-resident macrophages, are extensively involved in white matter MS pathology, but are also implicated in gray matter pathology, similarly to in other neurodegenerative diseases where there is synaptic, axonal, and neuronal degeneration. Microglia display regional heterogeneity within the CNS, which reflects their highly plastic nature and their ability to deliver context-dependent responses tailored to the demands of their microenvironment. Therefore, microglial roles in the MS gray matter in part reflect and in part diverge from those in the white matter. The present review summarizes current knowledge of microglial involvement in gray matter changes in MS, in demyelination, synaptic damage and neurodegeneration, with evidence implicating microglia in pathology, neuroprotection and repair. As our understanding of microglial physiology and pathophysiology increases, we describe how we are moving toward potential therapeutic applications in MS, harnessing microglia to protect and regenerate the CNS.

KEYWORDS
demyelination, gray matter, microglia, multiple sclerosis, neurodegeneration, synapse

1 | INTRODUCTION

The majority of the knowledge regarding microglial involvement in multiple sclerosis (MS) pathology comes from studying the white matter of MS patients and animal models. However, there is evidence for microglial regional heterogeneity within the central nervous system (CNS),[1] implying that there may be phenotypic differences between white and gray matter microglia. In addition, some features of gray matter pathology in MS are distinct from those typical to the white matter,[2] which might require or dictate different microglial responses. Both detrimental and beneficial roles have been reported for microglia in the literature, due to their highly plastic nature, allowing them to deliver tailored context-dependent responses. This review will present evidence supporting both damaging and beneficial microglial functions and underscore the highly complex interplay between pathological and restorative processes in MS gray matter. Here, we have chosen to focus on microglia only rather than other myeloid cells. The role of invading monocyte-derived macrophages is already well-described in CNS health and disease,[3-5] and although there is increasing interest in non-parenchymal CNS macrophages, it is difficult to separate out

their function as these were hitherto indistinguishable from microglia. Thus, some roles currently attributed to microglia may be, at least in part, executed by perivascular, meningeal, and choroid plexus macrophages. The development of novel tools that distinguish between microglia and non-parenchymal CNS macrophages[6] is likely to uncover their functions in MS gray matter in the future.

1.1 | What is MS?

MS is a chronic inflammatory neurodegenerative disease of the human CNS, of putative autoimmune aetiology, thought to be caused by complex interplay between genetic susceptibility and environmental risk factors.[7] The main pathological hallmark of MS is the presence of areas of demyelination, termed lesions, disseminated in time and space throughout the white and gray matter of the brain, spinal cord and optic nerve.[8] Usually, MS initially presents as a relapsing-remitting disease, in which bouts of demyelination manifest clinically as episodes of neurological dysfunction followed by spontaneous partial or complete recovery. Over time, the majority of MS patients transition into the secondary progressive phase of the disease, characterized by progressive and permanent accumulation of neurological disability.[8] Multiple disease-modifying treatments are available for the relapsing-remitting phase and aim to reduce inflammatory demyelinating activity, but their efficacy at preventing disability progression remains questionable.[9] MS research is hampered by the heterogeneous nature of the disease, as lesion pathology, clinical course and response to disease-modifying treatments vary greatly between patients, and are not accurately reproduced in preclinical models.[10]

1.2 | The importance of gray matter pathology in MS

The CNS is composed of two types of tissue: the white matter and the gray matter. The white matter is rich in neuronal axons and derives its name from the light color of the lipid-rich myelin sheaths surrounding the axons. In the gray matter, neuronal somata, dendrites and synapses predominate. As MS is characterized by the inflammatory destruction of myelin sheaths, it has been traditionally regarded as a disease of the white matter, where the majority of myelinated axons are concentrated. However, many of the axons originating from and terminating on neuronal somata in gray matter are myelinated, and so are also affected by demyelination in MS. Gray matter MS pathology involves demyelination, synaptic damage and neurodegeneration.[2] Demyelination can be repaired to some extent by the restoration of lost myelin sheaths around axons, termed remyelination; on the contrary, neurodegeneration in the gray matter appears irreversible, is prominent from the earliest stages of MS and is the substrate of a large proportion of permanent disability experienced by MS patients.[11,12] Furthermore, existing disease-modifying treatments can reduce white matter lesion load but seem rather limited in their ability to significantly reduce or prevent gray matter neurodegeneration.[13]

Significance statement

Gray matter pathology in multiple sclerosis is of great current interest as recent advances in live brain imaging reveal that this is present from the earliest disease stages, and, unlike white matter pathology, is significantly correlated with disability. Microglia, resident immune cells of the brain, are the first line of defense, with heterogeneity of their responses depending on whether they reside in gray or white matter. Understanding microglial responses to gray matter-specific cues will shed light on multiple sclerosis pathological processes and guide therapeutic strategies targeting the gray matter for neuroprotection—an unmet need to avoid and treat disability.

1.3 | Microglia are involved in MS pathology

Microglia are the only resident myeloid cells in CNS parenchyma in homeostasis, cooperating with the CNS border-associated macrophages.[14] They constantly survey their microenvironment through their dynamic processes, monitoring the proper function of neurons, axons and dendrites, and phagocytosing dying cells and cellular debris to maintain a healthy milieu.[15] In the neuroinflammatory MS environment, they can secrete proinflammatory cytokines and chemokines, promote oxidative stress and recruit peripheral immune cells across a compromised blood-brain barrier (BBB) into the CNS.[7] They are also paramount to the resolution of inflammation, as they phagocytose debris from myelin and cellular destruction, thus clearing the damage and creating an environment permissive to regeneration.[16] In addition, they secrete trophic factors for specialized adult stem cells, the oligodendrocyte precursor cells (OPCs), which migrate to areas of demyelination, differentiate into oligodendrocytes and remyelinate denuded axons.[17] Microglial functions in MS have overwhelmingly been investigated in white matter, which has traditionally been the focus of MS research; however, research is now showing that gray matter-specific pathological processes in MS elicit context-dependent microglial responses that can differ from those in white matter.

2 | MICROGLIA ARE INVOLVED IN ALL ASPECTS OF GRAY MATTER PATHOLOGY IN MS

In the gray matter, the complex interplay between demyelination, inflammation and neurodegeneration remains poorly understood. In late-stage MS, degeneration of chronically demyelinated axons and neurons is widespread in the gray matter,[18] correlating with gray matter

atrophy on magnetic resonance (MR) imaging, which is present even in early disease.[19] Demyelination can drive neurodegeneration,[20,21] but inflammation can also initiate degenerative mechanisms, including the structural and functional compromise of synapses, occurring prior to irreversible neuronal demise.[22] Evidence from other neurodegenerative diseases increasingly points to microglia as important effectors with both damaging and beneficial functions.[23] Gray matter microglia have been understudied, partly as microglial responses may differ between different disease stages, gray matter lesion pathology stages and between heterogeneous patients with different disease courses which cannot be easily reproduced in animal models. Here, we summarize findings of gray matter microglia in human MS and animal models, and speculate how much white matter microglial responses in MS and other neurodegenerative pathologies may inform us about gray matter microglial responses in MS. The absence of some evidence serves to strengthen the case for more research into gray matter-specific MS pathology. The following sections will explore the multifaceted microglial involvement in all three major aspects of MS-associated gray matter damage: demyelination, synaptic pathology, and neurodegeneration.

2.1 | Gray matter demyelination

Gray matter demyelination had been reported already by 1962,[24] but technological limitations in MR imaging obscured the true extent of its involvement in MS. It is now appreciated that almost all MS patients experience gray matter demyelination including in the cortex, hippocampus, thalamus, cerebellum, and spinal cord.[25] Permanent motor, cognitive and neuropsychiatric deficits have been proposed to at least partly have their pathological substrate in gray matter demyelination with the degree of dysfunction correlating better with gray rather than white matter demyelination.[26]

Functional deficits caused by demyelination may be resolved to various degrees by remyelination. Classically, remyelination is executed by OPCs, the adult stem cells of the CNS, but recent evidence suggests that mature surviving oligodendrocytes also contribute to this process.[27,28] In the cortical experimental autoimmune encephalomyelitis (EAE) and cuprizone mouse models of MS, gray matter remyelination is faster and more efficient than in white matter,[29,30] and in the human MS cortex, ultrastructural examination suggests that seemingly non-demyelinated (normal-appearing) gray matter may in fact be extensively remyelinated.[31] Whether the apparent increased propensity of the gray matter to remyelinate is a result of different regenerative mechanisms than in white matter, or whether similar repair processes may have greater efficiency due to a more permissive environment remains unknown.

2.1.1 | Microglial contribution to gray matter demyelination

In gray matter MS lesions seen on postmortem tissue, inflammation is less prominent than in white matter lesions, with less lymphocyte infiltration, complement deposition and fewer microglia/myeloid cells,[18] although biopsy material suggests that these feature more prominently in early disease.[32] However, even in postmortem tissue in chronic MS, inflammatory infiltrates consisting of T cells, B cells and plasma cells aggregate in the leptomeninges near subpial cortical and cerebellar gray matter lesions,[33] and in perivascular areas in deep gray matter,[34] surrounded by parenchymal activated microglia. These observations have led to the hypothesis that soluble mediators diffuse from meningeal or perivascular inflammatory aggregates into the gray matter to initiate demyelination with the help of activated microglia. Interferon-γ and tumor necrosis factor (TNF)-α are two such candidates, as they are highly expressed in the meninges and are associated with microglial activation[33]; furthermore, their presence in the cortex of rats with EAE is sufficient to initiate demyelination.[29] In early-stage white matter lesions, microglia secrete proinflammatory cytokines that directly damage myelin sheaths and their supporting oligodendrocytes,[35] but we are unclear whether this also occurs for gray matter microglia.

Gray matter demyelination may also occur secondary to white matter demyelination causing local axonal damage which spreads via anterograde or retrograde degeneration to anatomically and functionally connected gray matter areas.[36] This distant neurodegeneration is thought to "prime" neighboring white matter microglia, so upon subsequent challenge their responses are potentiated.[37] It is speculated that this may occur similarly in gray matter areas functionally connected to white matter lesions, poising microglia to quickly respond to meningeal or perivascular inflammatory infiltrates by secreting myelinotoxic effectors initiating demyelination.[38]

2.1.2 | Microglia aid robust remyelination

The myeloid cells of the innate immune system are paramount to the healing process in response to injury in peripheral tissues such as skin and muscle. Similarly, in MS-associated CNS injury, microglia are not confined to a damaging role but are essential for remyelination. In the demyelinating white matter, digestion of myelin debris transforms microglia from a pro- to an anti-inflammatory phenotype that suppresses inflammation to allow regeneration.[39] This has previously been described as a switch from an M1 proinflammatory to an M2 pro-regenerative state, but this is clearly an oversimplification, as microglia can assume multiple states resembling a continuum between the two extremes. Therefore, researchers have attempted to use transcriptomic analysis, markers, and function to try and define these cells better (see below), while still recognizing that the same function, for example, myelin phagocytosis, can be either damaging (early) or beneficial (later) depending on timing with respect to lesion pathology.

Microglia abound in remyelinating white matter lesions,[40] where they express markers of anti-inflammatory cytokines and immunomodulatory molecules,[41] presumably helping to resolve inflammation. Chronic gray matter lesions are less inflammatory than their white matter counterparts, and have an increased propensity to remyelinate,[31] which might in part be attributed to gray matter

microglia more effectively resolving the damage. Normal-appearing gray matter microglia highly express genes involved in type-I interferon responses, suggesting that these cells may be more immune-vigilant than their white matter counterparts.[42] As type-I interferon signaling in T cells produces anti-proliferative responses at least in vitro,[43] this raises the intriguing yet highly speculative possibility that immune-vigilant gray matter microglia may dampen CNS-infiltrating T cells.

Following the resolution of inflammation, the interactions between microglia, oligodendrocytes and their precursors in white matter lesions have been extensively studied. Microglia aid OPC migration into white matter lesions, from which they have largely been depleted during demyelination,[44] and OPC differentiation into remyelinating oligodendrocytes is supported by microglia with a pro-regenerative phenotype.[45] Rodent data indicate that OPC migration and differentiation are more efficient in gray matter,[46] suggesting that gray matter microglia may also be more efficient in assisting these oligodendroglial responses leading to remyelination. Microglia in the MS gray matter highly express genes involved in iron handling, perhaps allowing them to clear iron released during demyelination, which participates in oxidative injury,[47] or to facilitate iron acquisition by oligodendrocytes, which is required for myelin synthesis.[42] Furthermore, microglia may directly guide and/or modify remyelination by eliminating ectopic myelin sheaths as in development,[48] revealing a novel direct role for microglia in controlling myelination.

To summarize, gray matter microglia in MS are implicated in inflammatory demyelination but may also aid successful remyelination (Figure 1), and therefore make an attractive research target for therapeutic manipulation.

2.2 | Synapse dysfunction

The gray matter of the CNS harbors the majority of neuronal synapses, where communicating neurons exchange information that flows from the axonal presynaptic terminal of the input neuron to the postsynaptic site on the soma, dendrites, or axon of the recipient neuron. Therefore, proper neuronal communication depends upon optimal synaptic function. In MS, structural and functional compromise of synaptic connections between neurons has been reported in gray matter areas and could account in part for the cognitive deficits of patients with MS.[49] Cognitive impairment may even precede the appearance of typical MS clinical symptoms,[50] suggesting that synaptic function may be particularly vulnerable to subclinical disease activity. Synaptic damage has also been suggested as a substrate for "silent" disease progression, where disease-modifying treatments prevent the appearance of white matter lesions but do not prevent the progressive accumulation of disability.[9] Synaptic compromise appears among the earliest pathological events across neurodegenerative diseases including Alzheimer's, Parkinson's disease and amyotrophic lateral sclerosis (ALS), prior to neuronal demise, suggesting that diverse degenerative stimuli converge to induce a common early pathological response in synapses.[22] In MS, findings suggestive of synaptic pathology fall into three categories: (a) reduction in synaptic density within gray matter regions, (b) functional disturbances of synaptic transmission, and (c) perturbations in neurotransmitter homeostasis. There is evidence implicating microglia in all of the above.

2.2.1 | Microglial involvement in synaptic pathology

Microglia are prime suspects as effectors of MS-associated synaptic loss, as they eliminate synaptic connections during developmental circuit refinement to ensure proper brain connectivity,[51] and aberrant reactivation of developmental pruning mechanisms may contribute to degeneration-associated synaptic loss.[52] In MS, there is evidence of microglial engulfment and lysosomal digestion of synaptic components in postmortem hippocampus,[53] thalamus,[54] and cerebellar gray matter.[55] In EAE, synaptic components are found within microglia with a degeneration-associated phenotype before extensive pathology,[54] whereas in MS cortex in the absence of overt inflammation, microglia with a rod-like morphology have been suggested to execute synaptic stripping.[56] Inhibitory synapses are selectively reduced, compared with excitatory, in MS motor cortex[20] and hippocampus,[57] suggesting that they are especially vulnerable. As synaptic components are found within MS hippocampal microglia,[57] this suggests a role for microglia in this excitatory/inhibitory imbalance. Complement opsonization of target synapses may facilitate their recognition by microglia,[51] and increased complement deposition has been observed at hippocampal synapses in MS.[57] Notably, inhibition of synaptic complement tagging prevents their microglial engulfment and rescues functional deficits not only in EAE[54] but also in a mouse model of Alzheimer's disease with comparable synaptic pathology.[52] Changes in microglial phagocytosis may also affect other structures, as for example, in MS white matter, "do not-eat-me" signals that restrict microglial phagocytosis[58] show reduced expression.[59]

Microglia may also contribute to the functional compromise of synapses in MS independent of their loss.[60] In vivo in MS patients, positron emission tomography (PET) has confirmed the presence of activated microglia in the hippocampus[61] and thalamus,[62] and proinflammatory microglial cytokines impair synaptic transmission in the hippocampus of EAE mice.[63] In cerebrospinal fluid (CSF) isolated from MS patients high concentrations of IL-1β were capable of altering synaptic excitability in ex vivo rodent brain slices,[64] and incubation of healthy rodent brain slices with pre-activated microglia was sufficient to cause synaptic functional changes.[65] In EAE-affected mouse hippocampus, microglia increase their production of reactive oxygen species (ROS), leading to oxidative stress and impairment and/or loss of synaptic function.[66]

Finally, microglia-derived proinflammatory mediators may impair synaptic transmission by perturbing neurotransmitter homeostasis. In postmortem MS cortex, focal reduction of transporters that maintain physiological synaptic glutamate levels is observed in areas of microglial activation.[67] In EAE, IL-1β, TNF-α and ROS produced by activated

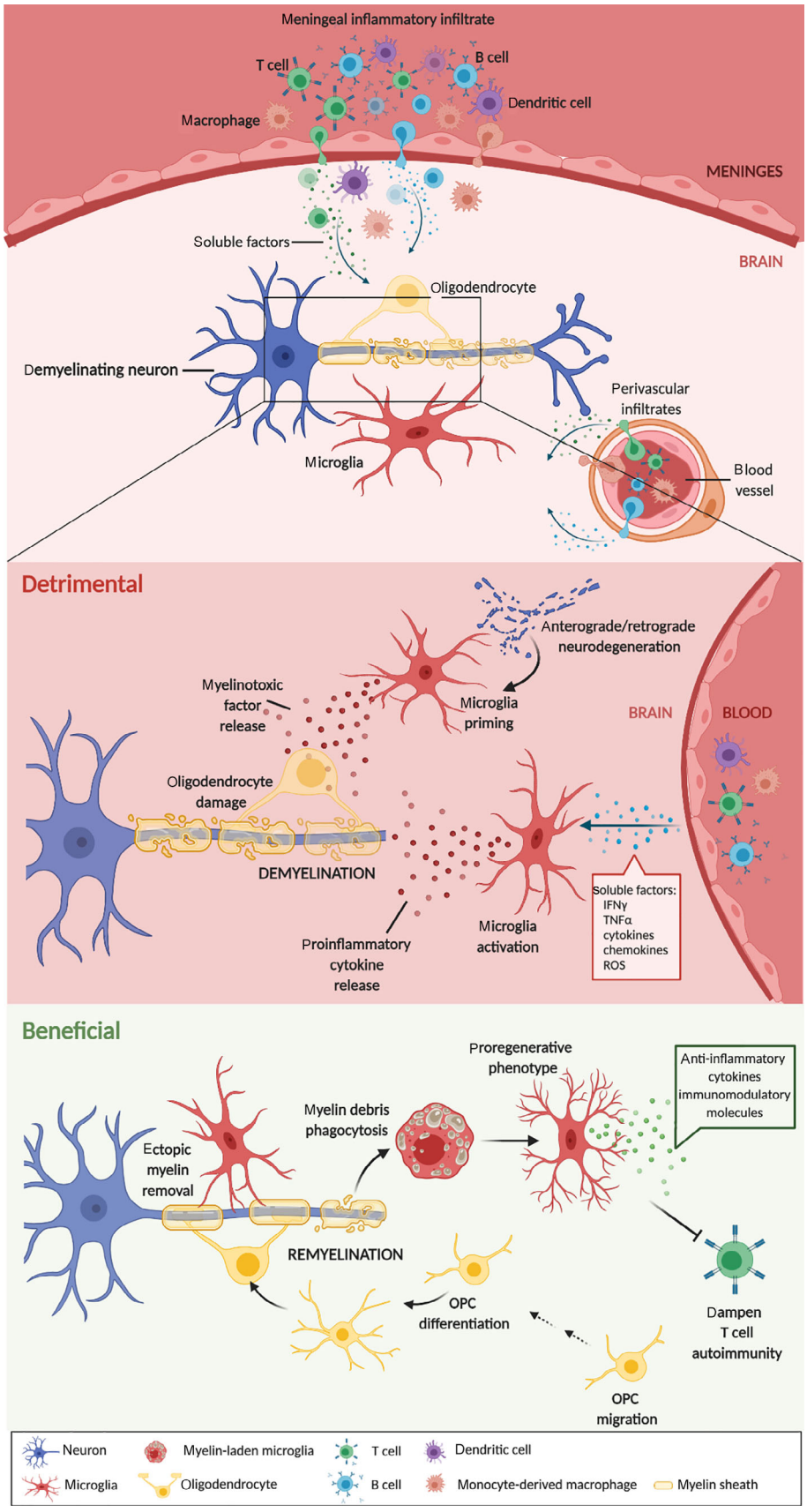

FIGURE 1 Model of how microglia may be involved in gray matter demyelination and remyelination. As yet unidentified soluble mediators diffuse from meningeal and perivascular inflammatory infiltrates (consisting of T and B lymphocytes, plasma cells, dendritic cells, and monocyte-derived macrophages) into the gray matter, activating resident microglia. Possible candidates include IFN-γ, TNF-α, proinflammatory cytokines and chemokines, and ROS. Activated microglia at least in early disease may secrete proinflammatory mediators directly damaging myelin sheaths and the oligodendrocytes that produce and maintain them, thus initiating demyelination. Gray matter demyelination may also arise secondary to demyelination in anatomically and functionally connected white matter areas. White matter axonal damage may also spread via anterograde/retrograde degeneration to the gray matter, where it may cause microglial "priming." Primed microglia may secrete myelinotoxic factors and thus participate in the initiation of demyelination. Microglia may also exert pro-regenerative functions supporting gray matter remyelination by removing myelin debris and transforming to a pro-regenerative phenotype as in white matter. Gray matter microglia may also dampen CNS-infiltrating T cell responses. Microglia may also directly guide and/or modify remyelination by eliminating ectopic myelin sheaths to ensure the accuracy of remyelination. IFN, interferon; MS, multiple sclerosis; OPC, oligodendrocyte precursor cell; ROS, reactive oxygen species; TNF, tumor necrosis factor

microglia can suppress the expression of neuronal and astrocytic glutamate transporters causing synaptic glutamate accumulation[68] and contributing to excitotoxicity, as discussed below.

2.2.2 | Microglia protect synaptic health

Although the removal of synapses or muting of their function may appear initially destructive, it may serve a compensatory purpose, aiming to protect surviving synapses, maintain network accuracy and restore circuit function. Injured neurons may instruct microglia to clear damaged synapses so that only healthy synapses remain in circuits.[69] In acute EAE, synaptic sites express apoptotic signals prior to neuronal loss and preceding their microglial removal.[65] During cortical inflammatory demyelination, microglial phagocytosis of synaptic structures is preceded by localized Ca^{2+} accumulation in dendritic shafts and spines, suggestive of synaptic dysfunction.[70] Microglial elimination of compromised synapses may also protect surrounding surviving synapses from the leakage of toxic amounts of glutamate and from the spread of oxidative stress.[71] The engulfment of apoptotic synaptic material may also transform microglia to an anti-inflammatory phenotype, as has been reported for macrophages in vitro, potentially dampening inflammation and aiding regeneration.[72]

Excitatory synapse removal by microglia may also dampen neuronal activity[73] at a time of circuit vulnerability. In EAE, motor function recovery is associated with reversible microglial displacement of synaptic contacts during acute disease, when the risk of excitotoxicity is high, followed by rapid re-apposition of pre- and post-synaptic elements during remission.[74] Additionally, upon inflammatory stimulation, microglial ROS can reduce the strength of excitatory synaptic transmission,[75] suggesting that MS neuroinflammation may induce a similar microglial response to weaken synaptic excitability and protect network hyperactivation. Furthermore, microglia-mediated dampening of energy-consuming synaptic transmission may help neurons channel their resources toward survival and recovery.

A perhaps initially counter-intuitive mechanism of microglial neuroprotection involves the temporary uncoupling of the pre- and post-synaptic components of inhibitory synapses,[76] with microglial processes closely apposing intact neuronal somata and dendrites seen in MS cortical lesions.[18] However, at least in mice, this can mediate neuroprotection by enhancing the activation of N-methyl-D-aspartate glutamate receptors and the transcription of pro-survival genes in neurons.[77] Microglia also guide the activity-dependent switching of post-synaptic structures between neighboring presynaptic inputs,[78] which if it also occurred in MS may also relocate post-synapses from compromised to nearby healthy presynaptic structures.

Finally, microglia may also aid synaptic regeneration. Microglia are indispensable for synapse formation and maturation during CNS plasticity, as they induce structural formation of post-synaptic structures,[79] secrete synaptotrophic factors including brain-derived neurotrophic factor (BDNF),[80] and modulate the perisynaptic extracellular matrix.[81] Perhaps following MS synaptic damage, microglia may recapitulate these mechanisms for regeneration of compromised synapses and support their functional maturation.

To summarize, gray matter microglia are implicated in MS-associated synaptic pathology, by directly eliminating synaptic connections and altering synaptic function through releasing inflammatory mediators. However, synaptic elimination by microglia, in at least preclinical models, may also exert protective or restorative effects. Further research is required to investigate whether gray matter microglia may modulate synaptic function to impart neuroprotection in MS (Figure 2).

2.3 | Neurodegeneration

Neurodegeneration is the progressive structural and functional compromise of neuronal somata, axons and dendrites, resulting in neuronal death and irreversible neurological dysfunction in patients with long-standing MS.[2] Pathological findings of neurodegeneration in MS gray matter include axonal swellings, dendritic and axonal transections and neuronal apoptosis.[18] Neurodegenerative mechanisms may be at work from the earliest stages of MS, even sometimes when neurological symptoms are not yet accompanied by radiological evidence supporting an MS diagnosis.[19] Recently, evidence for an MS prodrome has emerged, uncoupling the biological from the clinical onset of the disease, with increased serum levels of an axonal marker of degeneration appearing years before symptom onset.[82]

The MS inflammatory milieu may drive neurodegeneration, via neurotoxic molecules, including cytokines, ROS and glutamate produced by immune cells. ROS can induce cell death via oxidation of cellular components and mitochondrial damage, causing energy deficiency and activation of apoptotic and necrotic cascades.[83] Excess extracellular glutamate can cause neurodegeneration through excitotoxic mechanisms, during which sustained neuronal hyperexcitation precipitates neurotoxicity.[21] However, the relative inefficiency of immunomodulatory drugs in halting neurodegeneration, and as in chronic MS neurodegeneration proceeds behind an intact BBB, suggest that neurodegeneration may be in part uncoupled from peripheral immune mechanisms. CNS-resident microglia may be prime effectors of degenerative damage and are frequently found around areas of such damage.

2.3.1 | Microglia participate in neurodegeneration

Microglia with a phagocytic phenotype are found in close apposition to neuronal perikarya in areas of neuronal loss in cortex,[33] and PET in MS patients has revealed a correlation between higher cortical microglial load, cortical atrophy and reduced cognitive performance.[84] Microglia expressing glutamate- and ROS-synthesizing enzymes are found around dystrophic axons and neurons containing oxidized phospholipids in cortical MS lesions,[85,86] and microglia-derived ROS can lead to degeneration even of non-demyelinated axons.[87] Recent transcriptomic analysis has confirmed oxidative stress and mitochondrial demise within highly vulnerable neurons in upper layers of the demyelinated cortex.[88]

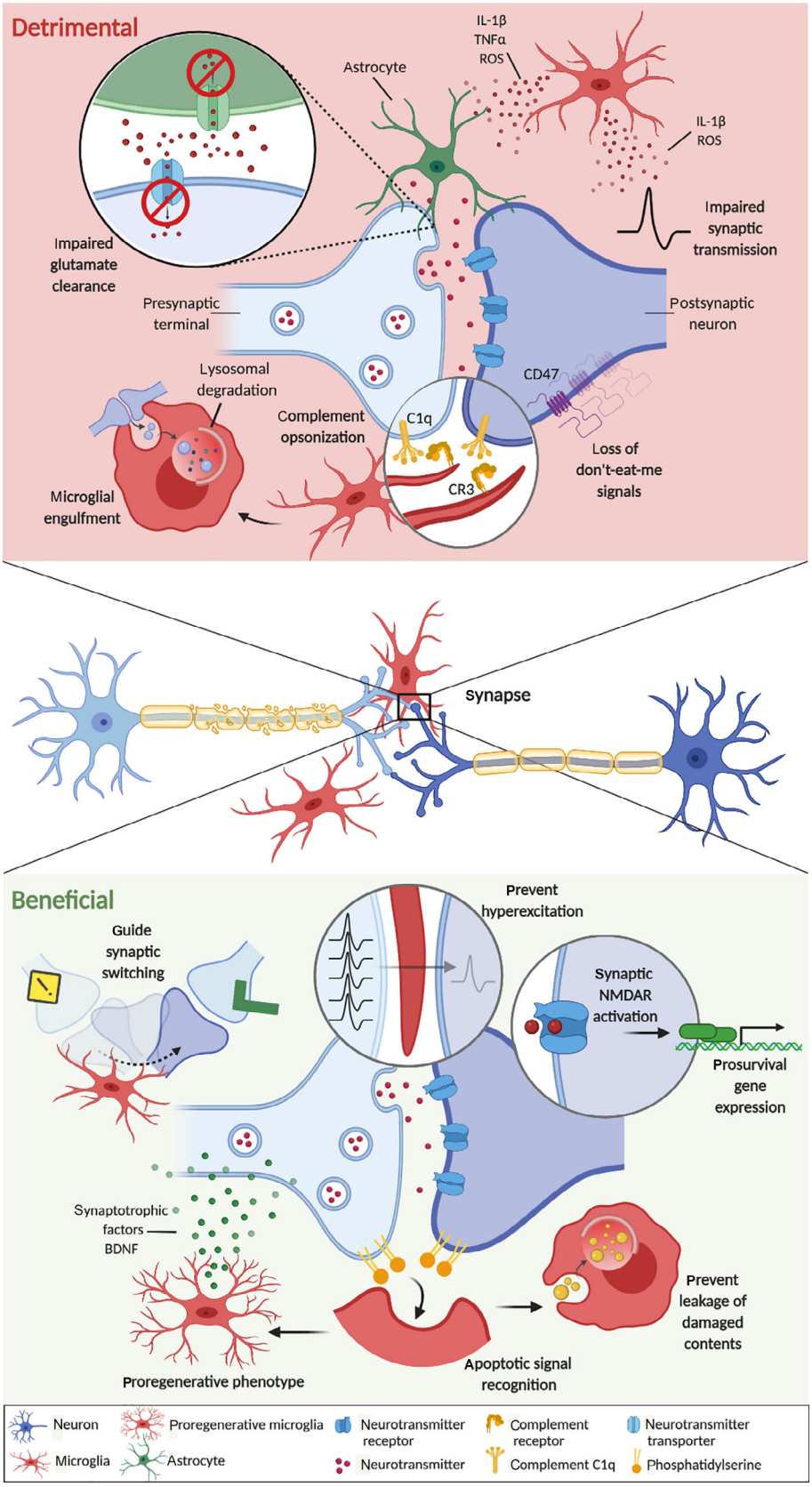

FIGURE 2 Model of how microglia may contribute to synaptic pathology but also protect and restore synaptic health. In MS gray matter lesions, synaptic structures are opsonized with complement components including C1q, which allows their microglial recognition via CR3, followed by their phagocytic elimination and lysosomal digestion within microglia. The loss of "do not-eat-me" signals, including CD47, from synaptic sites facilitates their microglial elimination. Activated microglia secrete proinflammatory cytokines including IL-1β and ROS, which impair synaptic transmission, suppressing the expression of glutamate reuptake transporters on neurons and astrocytes, leading to glutamate accumulation in the synaptic cleft and downstream excitotoxic synaptic damage. However, elimination of synapses following their externalization of apoptotic signals like phosphatidylserine may be beneficial, preventing leakage of toxic amounts of glutamate and ROS to neighboring healthy synapses, while also maintaining network accuracy via the prompt removal of compromised dysfunctional synapses. Excitatory synapse displacement by microglial processes may also dampen neuronal activity, preventing hyperexcitation, and subsequent excitotoxicity. Microglial displacement of inhibitory synapses may enhance synaptic NMDAR activation and downstream transcription of pro-survival genes, imparting neuroprotection. Microglia with a pro-regenerative phenotype may also promote synaptic regeneration and support their functional maturation via secreted synaptotrophic factors including BDNF. BDNF, brain-derived neurotrophic factor; CD, cluster of differentiation; CR, complement receptor; IL, interleukin; MS, multiple sclerosis; NMDAR, N-methyl-D-aspartate receptor; ROS, reactive oxygen species; TNF, tumor necrosis factor

Neurodegenerative pathology is comparable between MS gray matter, Alzheimer's and ALS, and this is reflected in elements of a degeneration-associated microglial signature being shared, at least at the transcript level, across these conditions. This signature is characterized by upregulation of proinflammatory transcripts, including *Clec7a*, *Gpnmb*, *Spp1*,[89,42,90] some (but not all) of which have been

confirmed also at the protein level.[54] Microglial phagocytosis of apoptotic neurons induces this disease-associated phenotype in mouse,[89] which is consistent with the hypothesis that neuronal injury from demyelination may precipitate self-sustaining neurodegenerative responses from CNS-resident cells.[7] Vulnerable neurons in the MS cortex upregulate transcripts for self-antigen presentation,[88] perhaps also making them targets for elimination by microglia. The reported association between thalamic microglial activation and cortical atrophy in MS patients on PET[84] further suggests a link between microglia and the spread of neurodegenerative pathology along neuronal connections.

Microglia do not act in isolation but communicate with other glia including astrocytes, and dysfunctional microglia-astrocyte crosstalk may contribute to MS neurodegeneration. Microglial inflammatory mediators, including TNF-α and IL-1α, can transform astrocytes into a neurotoxic phenotype, abundant in white matter MS lesions closely associated to activated microglia,[91] and this phenotype of astrocyte was also identified by transcriptomic analysis of MS gray matter as well as white.[92] In EAE, inhibition of the microglial inflammasome pathway prevented neurotoxic conversion of astrocytes, and rescued hippocampal neuropathology and related cognitive deficits,[93] emphasizing the importance of this crosstalk. The role of astrocytes in MS has been comprehensively reviewed very recently[94] and will not be covered further here.

2.3.2 | Microglia mediate neuroprotection

However, microglia also act to protect the host from insult, responding to danger signals to eliminate them and restore homeostasis. Prompt removal of irreversibly damaged neurons may preserve network function and prevent damage to neighboring healthy tissue. Description of these effects in white or gray matter separately is lacking in human or mouse, but following cuprizone-induced demyelination, which affects both gray and white matter, inhibition of microglial phagocytosis of damaged axons generally exacerbates axonal pathology, prolongs neuroinflammation and impairs remyelination,[95] whereas global enhancement of microglial phagocytosis reduces axonal damage in spinal cord EAE.[96] In vitro, microglial phagocytosis of apoptotic neurons before they lose their membrane integrity prevents release of proinflammatory cell death products,[71] and neuronal antigenic debris. This may be of importance in MS, as neuronal antigens such as TAG-1 may provoke a secondary autoimmune attack.[97] Microglia also phagocytose viable but stressed neurons, following downregulation of their "do not-eat-me" signals.[98]

The degeneration-associated microglial transcriptomic signature described in mouse models of several neurodegenerative diseases[89] has not been dissected into responses of microglia from gray and white matter. The signature includes genes that overlap with both classical M1-proinflammatory and M2-proregenerative markers,[89] suggesting that microglia in these models may play both roles. In response to experimental stroke, microglia promote neurogenesis, neural stem cell proliferation and differentiation,[99] and this maybe similar at least in chronic MS white matter lesions, as increased numbers of immature neurons are associated with hypertrophic microglia.[100] Furthermore, microglia may support axonal regeneration during compensatory network reorganization in MS, such as by guiding axonal pathfinding—similar to during development[101]—as they associate with sprouting axonal spheroids in MS tissue.[102] Furthermore, in human postmortem ischemic cortex, neuronal survival is associated with increased coverage of neuronal somata by microglia,[103] suggesting physical protection. In addition, in cortical mouse slices microglia enwrap artificially stimulated hyperactive axons, halting the spread of hyperactivity toward the soma and preventing excitotoxic neuronal death.[104] However, we recognize that these putative mechanisms by which gray matter microglia may aid neuroprotection in MS are as yet unproven.

To summarize, microglia in MS gray matter participate in neuronal, axonal, and dendritic damage that drive neurodegenerative pathology. However, evidence now suggests that microglia in MS (generally, but including those in gray matter) also may protect neuronal health, support proper network function, and participate in regenerative efforts, and we have speculated on mechanisms by which they do this (Figure 3).

3 | FUTURE DIRECTIONS TO UNDERSTAND THE ROLE OF GRAY MATTER MICROGLIA IN MS

3.1 | Dissecting the roles of different myeloid cells and their interactions with immune cells

The increasingly appreciated heterogeneity of microglial responses to disease is opening up new therapeutic directions but it also presents important challenges. Novel mouse transgenic lines now allow precise tracking and manipulation of microglia[105,106] and other CNS myeloid cells[6] with high specificity, and these will help us to understand both microglia-specific responses in the gray (and white) matter, and their interactions with CNS border-associated macrophages and peripheral monocyte-derived macrophages, which are of great importance in MS where CNS communication with the periphery remains poorly understood. Microglial interactions with adaptive immune cells, including T cells, while paramount to MS pathogenesis, are also necessary in maintaining microglial homeostasis and their interruption may lead to microglial dysfunction.[107] This raises important concerns in MS research, where adaptive immune cells are suppressed/eliminated by disease-modifying treatments, which may negatively impact microglial function, and raises some urgency to understand this better.

3.2 | Heterogeneity of human myeloid cells in MS gray vs white matter

Recent technical advances in single-cell/nuclei methods including RNA sequencing[90] and mass cytometry[108] allow unbiased, high-throughput

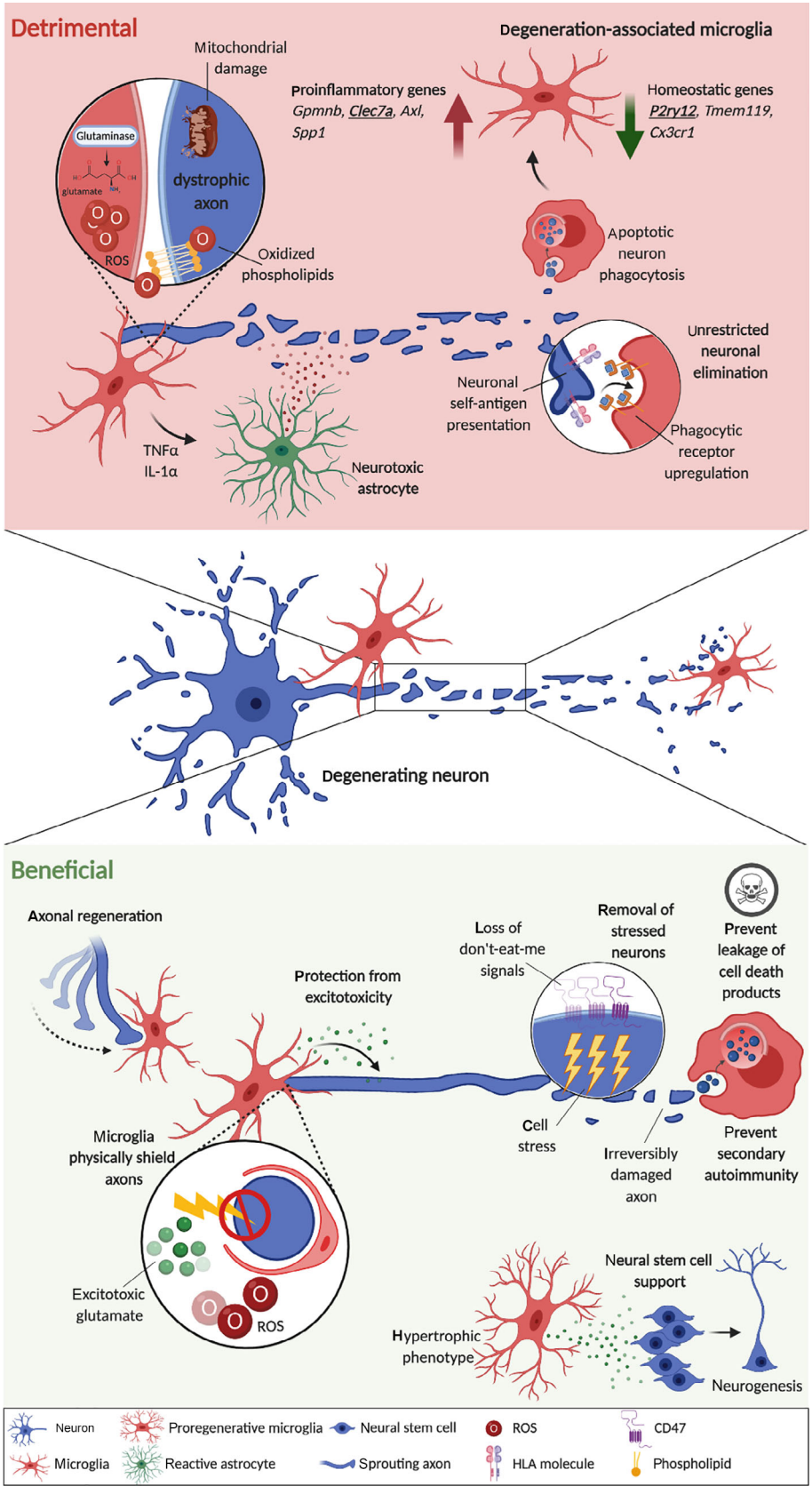

FIGURE 3 Model of how microglia may participate in neurodegeneration and neuroprotection. In MS white matter, a degeneration-associated microglial signature has been identified, induced by apoptotic neuron digestion, which includes downregulation of homeostatic and upregulation of proinflammatory genes. This degeneration-associated signature awaits validation in the gray matter—but already validated transcripts are underlined. These microglia may upregulate phagocytic receptor expression, which together with the upregulation of transcripts for self-antigen presentation by neurons, including HLA molecules, may lead to unrestricted neuronal elimination. Microglia overexpressing glutamate- and ROS-synthesizing enzymes associate with dystrophic neurons containing oxidized phospholipids and damaged mitochondria. Astrocytes with a neurotoxic reactive phenotype abound in MS gray matter lesions and can be induced by proinflammatory molecules released by activated microglia. However, the degeneration-associated microglial signature includes genes that overlap with a pro-regenerative phenotype, suggesting a potential beneficial role. Microglia may eliminate stressed neurons, reducing the release of harmful stress products and the accumulation of immunogenic neuronal debris, thus protecting nearby healthy neurons. Microglia may also support immature neurons/neurogenesis and axonal regeneration in MS lesions, similar to their developmental role, and may physically enwrap axons to shield them from an excitotoxic environment and prevent the spread of hyperactivity. HLA, human leukocyte antigen; IL, interleukin; MS, multiple sclerosis; ROS, reactive oxygen species; TNF, tumor necrosis factor

characterization of spatial and temporal heterogeneity of microglia in homeostasis and disease. Profiling microglia describes the diverse responses of these cells, but also gives us information about the context, environmental cues and pathways that elicit these responses, in health and disease. Regional variation in microglial transcriptomes has been described in mouse[109] and human,[90,108] but a comprehensive

white-gray matter comparison is lacking at the single-cell level. In human MS and mouse models, single-cell microglial profiling has mainly been performed in white matter,[110] or using mixed gray-white matter tissue,[90] which so far has hindered the identification and characterization of microglial populations specific to pathological gray matter. Future research in this area will help uncover differences in identity and function of gray matter microglia and dissect their roles in MS destructive and reparative processes. Novel technologies, such as CITE-seq that allows simultaneous readouts at the RNA and protein level,[111] ATAC-seq that informs on chromatin accessibility and thus on regulatory mechanisms guiding microglial identity, and spatial transcriptomic methods that preserve information on the spatial relationship between cells,[112] will be of critical significance. Identifying gray matter-specific homeostatic and pathological microglial clusters will pave the way to specifically targeting these populations to restore homeostasis and enhance regeneration.

3.3 | Human stem cell-derived preclinical models to study microglia in MS

A key outstanding issue in MS research (as with other diseases) is the human translatability of findings in animal models. Although murine and human microglia share a core expression profile and display comparable responses to demyelinating pathology,[90] human microglia display greater heterogeneity and a more immune-vigilant signature in homeostasis.[113] Of relevance to MS, human microglia are enriched for gene transcripts involved in crosstalk with the adaptive immune system,[114] but also in DNA repair/cell longevity and anti-inflammatory pathways.[113] Cross-species divergence is potentiated with aging, with reduced expression of genes involved in sensing the microenvironment, cell motility and migration in human but not in mouse microglia.[114] This divergence raises important concerns for research in neurodegeneration and other diseases of aging, suggesting that human-specific pathologies may not be recapitulated in commonly used models.

The differentiation of microglia from human induced pluripotent stem cells (iPSCs) has opened new research opportunities to elucidate human-specific physiology and pathology.[115,116] iPSC-derived microglia-like cells are amenable to genetic manipulations, enabling functional studies of newly identified human genes of interest, including those lacking murine orthologues. Co-culture with neurons and the addition of soluble factors found in CNS milieu direct iPSC-derived microglia toward a homeostatic state resembling CNS-resident cells.[117] Furthermore, iPSC-derived microglia can be incorporated into 3D brain organoids, where they interact with neurons, astro- and oligodendroglia, and respond to challenge similar to CNS-resident microglia.[117] Brain region-specific organoids may recapitulate aspects of the CNS region-specific microenvironment and thus help shed light on context-dependent cues that shape microglial identity. Studying microglia derived from iPSCs from MS patients may help elucidate microglia-intrinsic and -extrinsic contributions to MS pathology, as has been described for Alzheimer's disease,[118] and perhaps explore some rudimentary gene-environment interactions in a reductionist context.

However, in in vitro models, iPSC-derived microglia are removed from their natural habitat and thus their homeostatic signature is altered. The generation of chimeric animal models, with transplanted iPSC-derived microglia-like cells may provide an invaluable boost in translational microglial research,[119,120] by allowing the study and manipulation of human microglia inside a physiological or pathological environment, albeit a rodent one. Already it is known that human iPSC-derived microglia retain their identity upon transplantation in the murine brain,[121] but findings need to be interpreted with caution, as the host organism immunodeficiency required to accept the xenotransplantation changes the CNS immunological environment, which may confound results. Furthermore, transplanted iPSC-derived microglia-like cells may be distinct from true CNS-resident microglia, due to differences in cell origin and environment[122]; this may confound research but also offer therapeutic advantages if their phenotype is advantageous.[123] Another exciting opportunity arises with direct reprogramming from adult cells, bypassing pluripotency, which may conserve transcriptomic signatures of age[124] and may allow the modeling of later-life human microglial responses within the limited lifespan of a laboratory rodent. Once the "signature" of gray matter microglia is established, acknowledging that these may also be heterogenous, then it may be possible to target iPSC differentiation into specific microglial subtypes, followed by xenotransplantation in rodent brains under physiological and pathological conditions to discover their functions and identify microglial populations with therapeutic potential.

However, therapeutic approaches targeting microglia in MS will need to be sophisticated due to their regional and temporal phenotypic and functional heterogeneity. Even the "disease-associated" microglial phenotype, which seems generic to several diseases at least in mice, when crudely targeted, may prevent beneficial microglial activation alongside the suppression of their harmful effects,[125] especially in a chronic disease such as MS, where different stages of the disease elicit different—and perhaps opposite—microglial responses, and identifying the optimal timing for possible intervention seems paramount. In MS, lesions across the CNS are not synchronized in their stage of pathology, further complicating matters. The importance of optimal timing for harnessing microglia-mediated neuroprotection was recently demonstrated in a brain injury model, where microglial repopulation stimulated neuroprotective pathways and improved functional outcomes only during the acute injury phase.[126] If we can understand the type and timing of optimal microglial therapeutic targeting, then nanotechnological approaches may help to selectively target harmful microglial types in areas of pathology while non-affected regions are spared. The discovery of easily accessible biomarkers of microglial phenotypes may also eventually allow real-time monitoring of treatment effects in the living human brain using techniques such as PET.[62] Already in liver cirrhosis, the safety and tolerability of autologous macrophage infusion following their in vitro polarization to an advantageous pro-regenerative phenotype have been demonstrated in a phase I clinical trial.[127] This provides an

exciting proof of concept for the feasibility of therapeutic neuroprotective microglial replacement in MS patients.

4 | CONCLUSION

MS gray matter microglia contribute to demyelination, synaptic and neuronal injury but are also implicated in inflammation resolution, synaptic remodeling, neuroprotection, and regeneration. Their highly dynamic nature allows microglia to change their gene expression on demand, so that there may be multiple different functional states to perform specific tasks in response to specific environmental cues. The ongoing advances in omics technologies are furthering our understanding of this spectrum of microglial states in homeostasis and disease. However, the conditions that guide microglia toward detrimental or beneficial phenotypes remain poorly understood in MS gray matter. Therefore, the challenge for the future lies in deciphering the context-specific responses of microglia and using that knowledge to harness their beneficial properties while suppressing their injurious reactions.

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CONFLICT OF INTEREST

A.W. declared funding from Roche for a postdoctoral scientist in her group for non-drug related research. The other author declared no potential conflicts of interest.

AUTHOR CONTRIBUTIONS

F.T., A.W.: review conceived and written; F.T.: figures made.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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REFERENCES

1. Grabert K, Michoel T, Karavolos MH, et al. Microglial brain region-dependent diversity and selective regional sensitivities to aging. *Nat Neurosci*. 2016;19(3):504-516. https://doi.org/10.1038/nn.4222.
2. Wegner C, Esiri MM, Chance SA, Palace J, Matthews PM. Neocortical neuronal, synaptic, and glial loss in multiple sclerosis. *Neurology*. 2006;67(6):960-967. http://n.neurology.org/content/67/6/960.abstract.
3. Prinz M, Priller J. The role of peripheral immune cells in the CNS in steady state and disease. *Nat Neurosci*. 2017;20(2):136-144. https://doi.org/10.1038/nn.4475.
4. Zia S, Rawji KS, Michaels NJ, et al. Microglia diversity in health and multiple sclerosis. *Front Immunol*. 2020;11:2895. https://doi.org/10.3389/fimmu.2020.588021.
5. Yamasaki R, Lu H, Butovsky O, et al. Differential roles of microglia and monocytes in the inflamed central nervous system. *J Exp Med*. 2014;211(8):1533-1549. https://doi.org/10.1084/jem.20132477.
6. Kim J-S, Kolesnikov M, Peled-Hajaj S, et al. A binary Cre transgenic approach dissects microglia and CNS border-associated macrophages. *Immunity*. 2021;54(1):176-190.e7. https://doi.org/10.1016/j.immuni.2020.11.007.
7. Dendrou CA, Fugger L, Friese MA. Immunopathology of multiple sclerosis. *Nat Rev Immunol*. 2015;15(9):545-558. https://doi.org/10.1038/nri3871.
8. Reich DS, Lucchinetti CF, Calabresi PA. Multiple sclerosis. *N Engl J Med*. 2018;378(2):169-180. https://doi.org/10.1056/NEJMra1401483.
9. University of California SFM-ET, Cree BAC, Hollenbach JA, et al. Silent progression in disease activity-free relapsing multiple sclerosis. *Ann Neurol*. 2019;85(5):653-666. https://doi.org/10.1002/ana.25463.
10. Lucchinetti C, Brück W, Parisi J, Scheithauer B, Rodriguez M, Lassmann H. Heterogeneity of multiple sclerosis lesions: implications for the pathogenesis of demyelination. *Ann Neurol*. 2000;47(6):707-717. https://doi.org/10.1002/1531-8249(200006)47:6<707::AID-ANA3>3.0.CO;2-Q.
11. Popescu V, Klaver R, Voorn P, et al. What drives MRI-measured cortical atrophy in multiple sclerosis? *Mult Scler J*. 2015;21(10):1280-1290. https://doi.org/10.1177/1352458514562440.
12. Filippi M, Preziosa P, Copetti M, et al. Gray matter damage predicts the accumulation of disability 13 years later in MS. *Neurology*. 2013;81(20):1759-1767. https://doi.org/10.1212/01.wnl.0000435551.90824.d0.
13. Hauser SL, Cree BAC. Treatment of multiple sclerosis: a review. *Am J Med*. 2020;133(12):1380-1390.e2. https://doi.org/10.1016/j.amjmed.2020.05.049.
14. Prinz M, Erny D, Hagemeyer N. Ontogeny and homeostasis of CNS myeloid cells. *Nat Immunol*. 2017;18(4):385-392. https://doi.org/10.1038/ni.3703.
15. Nimmerjahn A, Kirchhoff F, Helmchen F. Resting microglial cells are highly dynamic surveillants of brain parenchyma in vivo. *Science (80-)*. 2005;308(5726):1314-1318. https://doi.org/10.1126/science.1110647.
16. Kotter MR, Li W-W, Zhao C, Franklin RJM, Myelin Impairs CNS. Remyelination by inhibiting oligodendrocyte precursor cell differentiation. *J Neurosci*. 2006;26(1):328-332. https://doi.org/10.1523/JNEUROSCI.2615-05.2006.
17. Lloyd AF, Miron VE. The pro-remyelination properties of microglia in the central nervous system. *Nat Rev Neurol*. 2019;15(8):447-458. https://doi.org/10.1038/s41582-019-0184-2.
18. Peterson JW, Bö L, Mörk S, Chang A, Trapp BD. Transected neurites, apoptotic neurons, and reduced inflammation in cortical multiple sclerosis lesions. *Ann Neurol*. 2001;50(3):389-400. https://doi.org/10.1002/ana.1123.
19. Dalton CM, Chard DT, Davies GR, et al. Early development of multiple sclerosis is associated with progressive grey matter atrophy in patients presenting with clinically isolated syndromes. *Brain*. 2004;127(5):1101-1107. https://doi.org/10.1093/brain/awh126.
20. Zoupi L, Booker SA, Eigel D, et al. Selective vulnerability of inhibitory networks in multiple sclerosis. *Acta Neuropathol*. 2021;141:415-429. https://doi.org/10.1007/s00401-020-02258-z.
21. Friese MA, Schattling B, Fugger L. Mechanisms of neurodegeneration and axonal dysfunction in multiple sclerosis. *Nat Rev Neurol*. 2014;10(4):225-238. https://doi.org/10.1038/nrneurol.2014.37.
22. Wishart TM, Parson SH, Gillingwater TH. Synaptic vulnerability in neurodegenerative disease. *J Neuropathol Exp Neurol*. 2006;65(8):733-739. https://doi.org/10.1097/01.jnen.0000228202.35163.c4.
23. Salter MW, Stevens B. Microglia emerge as central players in brain disease. *Nat Med*. 2017;23(9):1018-1027. https://doi.org/10.1038/nm.4397.

24. Brownell B, Hughes JT. The distribution of plaques in the cerebrum in multiple sclerosis. *J Neurol Neurosurg Psychiatry*. 1962;25(4):315-320. https://doi.org/10.1136/jnnp.25.4.315.
25. Gilmore CP, Donaldson I, Bö L, Owens T, Lowe J, Evangelou N. Regional variations in the extent and pattern of grey matter demyelination in multiple sclerosis: a comparison between the cerebral cortex, cerebellar cortex, deep grey matter nuclei and the spinal cord. *J Neurol Neurosurg Psychiatry*. 2009;80(2):182-187. https://doi.org/10.1136/jnnp.2008.148767.
26. Geurts JJG, Calabrese M, Fisher E, Rudick RA. Measurement and clinical effect of grey matter pathology in multiple sclerosis. *Lancet Neurol*. 2012;11(12):1082-1092. https://doi.org/10.1016/S1474-4422(12)70230-2.
27. Bacmeister CM, Barr HJ, McClain CR, et al. Motor learning promotes remyelination via new and surviving oligodendrocytes. *Nat Neurosci*. 2020;23(7):819-831. https://doi.org/10.1038/s41593-020-0637-3.
28. Duncan ID, Radcliff AB, Heidari M, Kidd G, August BK, Wierenga LA. The adult oligodendrocyte can participate in remyelination. *Proc Natl Acad Sci*. 2018;115(50):E11807-E11816. https://doi.org/10.1073/pnas.1808064115.
29. Merkler D, Ernsting T, Kerschensteiner M, Brück W, Stadelmann C. A new focal EAE model of cortical demyelination: multiple sclerosis-like lesions with rapid resolution of inflammation and extensive remyelination. *Brain*. 2006;129(8):1972-1983. https://doi.org/10.1093/brain/awl135.
30. Bai CB, Sun S, Roholt A, et al. A mouse model for testing remyelinating therapies. *Exp Neurol*. 2016;283:330-340. https://doi.org/10.1016/j.expneurol.2016.06.033.
31. Albert M, Antel J, Brück W, Stadelmann C. Extensive cortical remyelination in patients with chronic multiple sclerosis. *Brain Pathol*. 2007;17(2):129-138. https://doi.org/10.1111/j.1750-3639.2006.00043.x.
32. Lucchinetti CF, Popescu BFG, Bunyan RF, et al. Inflammatory cortical demyelination in early multiple sclerosis. *N Engl J Med*. 2011;365(23):2188-2197. https://doi.org/10.1056/NEJMoa1100648.
33. Magliozzi R, Howell OW, Reeves C, et al. A gradient of neuronal loss and meningeal inflammation in multiple sclerosis. *Ann Neurol*. 2010;68(4):477-493. https://doi.org/10.1002/ana.22230.
34. Vercellino M, Masera S, Lorenzatti M, et al. Demyelination, inflammation, and neurodegeneration in multiple sclerosis deep gray matter. *J Neuropathol Exp Neurol*. 2009;68(5):489-502. https://doi.org/10.1097/NEN.0b013e3181a19a5a.
35. Bitsch A, Kuhlmann T, Da Costa C, Bunkowski S, Polak T, Brück W. Tumour necrosis factor alpha mRNA expression in early multiple sclerosis lesions: correlation with demyelinating activity and oligodendrocyte pathology. *Glia*. 2000;29(4):366-375. https://doi.org/10.1002/(SICI)1098-1136(20000215)29:4<366::AID-GLIA7>3.0.CO;2-Y.
36. Dziedzic T, Metz I, Dallenga T, et al. Wallerian degeneration: a major component of early axonal pathology in multiple sclerosis. *Brain Pathol*. 2010;20(5):976-985. https://doi.org/10.1111/j.1750-3639.2010.00401.x.
37. Ramaglia V, Hughes TR, Donev RM, et al. C3-dependent mechanism of microglial priming relevant to multiple sclerosis. *Proc Natl Acad Sci*. 2012;109(3):965-970. https://doi.org/10.1073/pnas.1111924109.
38. Lassmann H. Cortical lesions in multiple sclerosis: inflammation versus neurodegeneration. *Brain*. 2012;135(10):2904-2905. https://doi.org/10.1093/brain/aws260.
39. Lloyd AF, Davies CL, Holloway RK, et al. Central nervous system regeneration is driven by microglia necroptosis and repopulation. *Nat Neurosci*. 2019;22(7):1046-1052. https://doi.org/10.1038/s41593-019-0418-z.
40. Zrzavy T, Hametner S, Wimmer I, Butovsky O, Weiner HL, Lassmann H. Loss of "homeostatic" microglia and patterns of their activation in active multiple sclerosis. *Brain*. 2017;140(7):1900-1913. https://doi.org/10.1093/brain/awx113.
41. Psachoulia K, Chamberlain KA, Heo D, et al. IL4I1 augments CNS remyelination and axonal protection by modulating T cell driven inflammation. *Brain*. 2016;139(12):3121-3136. https://doi.org/10.1093/brain/aww254.
42. van der Poel M, Ulas T, Mizee MR, et al. Transcriptional profiling of human microglia reveals grey-white matter heterogeneity and multiple sclerosis-associated changes. *Nat Commun*. 2019;10(1):1139. https://doi.org/10.1038/s41467-019-08976-7.
43. Owens T, Khorooshi R, Wlodarczyk A, Asgari N. Interferons in the central nervous system: a few instruments play many tunes. *Glia*. 2014;62(3):339-355. https://doi.org/10.1002/glia.22608.
44. Zhao C, Fancy SPJ, Franklin RJM, French-Constant C. Up-regulation of oligodendrocyte precursor cell αV integrin and its extracellular ligands during central nervous system remyelination. *J Neurosci Res*. 2009;87(15):3447-3455. https://doi.org/10.1002/jnr.22231.
45. Dillenburg A, Ireland G, Holloway RK, et al. Activin receptors regulate the oligodendrocyte lineage in health and disease. *Acta Neuropathol*. 2018;135(6):887-906. https://doi.org/10.1007/s00401-018-1813-3.
46. Strijbis EMM, Kooi E-J, van der Valk P, Geurts JJG. Cortical remyelination is heterogeneous in multiple sclerosis. *J Neuropathol Exp Neurol*. 2017;76(5):390-401. https://doi.org/10.1093/jnen/nlx023.
47. Haider L, Simeonidou C, Steinberger G, et al. Multiple sclerosis deep grey matter: the relation between demyelination, neurodegeneration, inflammation and iron. *J Neurol Neurosurg Psychiatry*. 2014;85(12):1386-1395. https://doi.org/10.1136/jnnp-2014-307712.
48. Hughes AN, Appel B. Microglia phagocytose myelin sheaths to modify developmental myelination. *Nat Neurosci*. 2020;23(9):1055-1066. https://doi.org/10.1038/s41593-020-0654-2.
49. Di Filippo M, Portaccio E, Mancini A, Calabresi P. Multiple sclerosis and cognition: synaptic failure and network dysfunction. *Nat Rev Neurosci*. 2018;19(10):599-609. https://doi.org/10.1038/s41583-018-0053-9.
50. Amato MP, Hakiki B, Goretti B, et al. Association of MRI metrics and cognitive impairment in radiologically isolated syndromes. *Neurology*. 2012;78(5):309-314. https://doi.org/10.1212/WNL.0b013e31824528c9.
51. Schafer DP, Lehrman EK, Kautzman AG, et al. Microglia sculpt postnatal neural circuits in an activity and complement-dependent manner. *Neuron*. 2012;74(4):691-705. https://doi.org/10.1016/j.neuron.2012.03.026.
52. Hong S, Beja-Glasser VF, Nfonoyim BM, et al. Complement and microglia mediate early synapse loss in Alzheimer mouse models. *Science (80-)*. 2016;352(6286):712-716. http://science.sciencemag.org/content/352/6286/712.abstract.
53. Michailidou I, Willems JGP, Kooi EJ, et al. Complement C1q-C3-associated synaptic changes in multiple sclerosis hippocampus. *Ann Neurol*. 2015;77(6):1007-1026. https://doi.org/10.1002/ana.24398.
54. Werneburg S, Jung J, Kunjamma RB, et al. Targeted complement inhibition at synapses prevents microglial synaptic engulfment and synapse loss in demyelinating disease. *Immunity*. 2020;52(1):167-182.e7. https://doi.org/10.1016/j.immuni.2019.12.004.
55. Albert M, Barrantes-Freer A, Lohrberg M, et al. Synaptic pathology in the cerebellar dentate nucleus in chronic multiple sclerosis. *Brain Pathol*. 2017;27(6):737-747. https://doi.org/10.1111/bpa.12450.
56. van Wageningen TA, Vlaar E, Kooij G, Jongenelen CAM, Geurts JJG, van Dam A-M. Regulation of microglial TMEM119 and P2RY12 immunoreactivity in multiple sclerosis white and grey matter lesions is dependent on their inflammatory environment. *Acta Neuropathol Commun*. 2019;7(1):206. https://doi.org/10.1186/s40478-019-0850-z.

57. Ramaglia V, Dubey M, Malpede MA, et al. Complement-associated loss of CA2 inhibitory synapses in the demyelinated hippocampus impairs memory. *bioRxiv*. 2021;15.426022. https://doi.org/10.1101/2021.01.15.426022.
58. Lehrman EK, Wilton DK, Litvina EY, et al. CD47 protects synapses from excess microglia-mediated pruning during development. *Neuron*. 2018;100(1):120-134.e6. https://doi.org/10.1016/j.neuron.2018.09.017.
59. Koning N, Bö L, Hoek RM, Huitinga I. Downregulation of macrophage inhibitory molecules in multiple sclerosis lesions. *Ann Neurol*. 2007;62(5):504-514. https://doi.org/10.1002/ana.21220.
60. Mandolesi G, Gentile A, Musella A, et al. Synaptopathy connects inflammation and neurodegeneration in multiple sclerosis. *Nat Rev Neurol*. 2015;11(12):711-724. https://doi.org/10.1038/nrneurol.2015.222.
61. Colasanti A, Guo Q, Giannetti P, et al. Hippocampal neuroinflammation, functional connectivity, and depressive symptoms in multiple sclerosis. *Biol Psychiatry*. 2016;80(1):62-72. https://doi.org/10.1016/j.biopsych.2015.11.022.
62. Sucksdorff M, Matilainen M, Tuisku J, et al. Brain TSPO-PET predicts later disease progression independent of relapses in multiple sclerosis. *Brain*. 2020;143(11):3318-3330. https://doi.org/10.1093/brain/awaa275.
63. Nisticò R, Mango D, Mandolesi G, et al. Inflammation subverts hippocampal synaptic plasticity in experimental multiple sclerosis. *PLoS One*. 2013;8(1):e54666. https://doi.org/10.1371/journal.pone.0054666.
64. Rossi S, Furlan R, De Chiara V, et al. Interleukin-1β causes synaptic hyperexcitability in multiple sclerosis. *Ann Neurol*. 2012;71(1):76-83. https://doi.org/10.1002/ana.22512.
65. Centonze D, Muzio L, Rossi S, et al. Inflammation triggers synaptic alteration and degeneration in experimental autoimmune encephalomyelitis. *J Neurosci*. 2009;29(11):3442-3452. https://doi.org/10.1523/JNEUROSCI.5804-08.2009.
66. Di Filippo M, de Iure A, Giampà C, et al. Persistent activation of microglia and NADPH oxidase drive hippocampal dysfunction in experimental multiple sclerosis. *Sci Rep*. 2016;6(1):20926. https://doi.org/10.1038/srep20926.
67. Vercellino M, Merola A, Piacentino C, et al. Altered glutamate reuptake in relapsing-remitting and secondary progressive multiple sclerosis cortex: correlation with microglia infiltration, demyelination, and neuronal and synaptic damage. *J Neuropathol Exp Neurol*. 2007;66(8):732-739. https://doi.org/10.1097/nen.0b013e31812571b0.
68. Stampanoni Bassi M, Mori F, Buttari F, et al. Neurophysiology of synaptic functioning in multiple sclerosis. *Clin Neurophysiol*. 2017;128(7):1148-1157. https://doi.org/10.1016/j.clinph.2017.04.006.
69. Gunner G, Cheadle L, Johnson KM, et al. Sensory lesioning induces microglial synapse elimination via ADAM10 and fractalkine signaling. *Nat Neurosci*. 2019;22(7):1075-1088. https://doi.org/10.1038/s41593-019-0419-y.
70. Jafari M, Schumacher A-M, Snaidero N, et al. Phagocyte-mediated synapse removal in cortical neuroinflammation is promoted by local calcium accumulation. *Nat Neurosci*. 2021;24:355-367. https://doi.org/10.1038/s41593-020-00780-7.
71. Takahashi K, Rochford CDP, Neumann H. Clearance of apoptotic neurons without inflammation by microglial triggering receptor expressed on myeloid cells-2. *J Exp Med*. 2005;201(4):647-657. https://doi.org/10.1084/jem.20041611.
72. Fadok VA, Bratton DL, Konowal A, Freed PW, Westcott JY, Henson PM. Macrophages that have ingested apoptotic cells in vitro inhibit proinflammatory cytokine production through autocrine/paracrine mechanisms involving TGF-beta, PGE2, and PAF. *J Clin Invest*. 1998;101(4):890-898. https://doi.org/10.1172/JCI1112.
73. Badimon A, Strasburger HJ, Ayata P, et al. Negative feedback control of neuronal activity by microglia. *Nature*. 2020;586(7829):417-423. https://doi.org/10.1038/s41586-020-2777-8.
74. Marques KB, Santos LMB, Oliveira ALR. Spinal motoneuron synaptic plasticity during the course of an animal model of multiple sclerosis. *Eur J Neurosci*. 2006;24(11):3053-3062. https://doi.org/10.1111/j.1460-9568.2006.05184.x.
75. Zhang J, Malik A, Choi HB, Ko RWY, Dissing-Olesen L, MacVicar BA. Microglial CR3 activation triggers long-term synaptic depression in the hippocampus via NADPH oxidase. *Neuron*. 2014;82(1):195-207. https://doi.org/10.1016/j.neuron.2014.01.043.
76. Chen Z, Jalabi W, Shpargel KB, et al. Lipopolysaccharide-induced microglial activation and neuroprotection against experimental brain injury is independent of hematogenous TLR4. *J Neurosci*. 2012;32(34):11706-11715. https://doi.org/10.1523/JNEUROSCI.0730-12.2012.
77. Chen Z, Jalabi W, Hu W, et al. Microglial displacement of inhibitory synapses provides neuroprotection in the adult brain. *Nat Commun*. 2014;5(1):4486. https://doi.org/10.1038/ncomms5486.
78. Weinhard L, di Bartolomei G, Bolasco G, et al. Microglia remodel synapses by presynaptic trogocytosis and spine head filopodia induction. *Nat Commun*. 2018;9(1):1228. https://doi.org/10.1038/s41467-018-03566-5.
79. Miyamoto A, Wake H, Ishikawa AW, et al. Microglia contact induces synapse formation in developing somatosensory cortex. *Nat Commun*. 2016;7(1):12540. https://doi.org/10.1038/ncomms12540.
80. Parkhurst CN, Yang G, Ninan I, et al. Microglia promote learning-dependent synapse formation through brain-derived neurotrophic factor. *Cell*. 2013;155(7):1596-1609. https://doi.org/10.1016/j.cell.2013.11.030.
81. Nguyen PT, Dorman LC, Pan S, et al. Microglial remodeling of the extracellular matrix promotes synapse plasticity. *Cell*. 2020;182:388-403.e15. https://doi.org/10.1016/j.cell.2020.05.050.
82. Bjornevik K, Munger KL, Cortese M, et al. Serum neurofilament light chain levels in patients with presymptomatic multiple sclerosis. *JAMA Neurol*. 2020;77(1):58-64. https://doi.org/10.1001/jamaneurol.2019.3238.
83. Haider L, Fischer MT, Frischer JM, et al. Oxidative damage in multiple sclerosis lesions. *Brain*. 2011;134(7):1914-1924. https://doi.org/10.1093/brain/awr128.
84. Herranz E, Giannì C, Louapre C, et al. Neuroinflammatory component of gray matter pathology in multiple sclerosis. *Ann Neurol*. 2016;80(5):776-790. https://doi.org/10.1002/ana.24791.
85. Werner P, Pitt D, Raine CS. Multiple sclerosis: altered glutamate homeostasis in lesions correlates with oligodendrocyte and axonal damage. *Ann Neurol*. 2001;50(2):169-180. https://doi.org/10.1002/ana.1077.
86. Fischer MT, Wimmer I, Höftberger R, et al. Disease-specific molecular events in cortical multiple sclerosis lesions. *Brain*. 2013;136(6):1799-1815. https://doi.org/10.1093/brain/awt110.
87. Nikić I, Merkler D, Sorbara C, et al. A reversible form of axon damage in experimental autoimmune encephalomyelitis and multiple sclerosis. *Nat Med*. 2011;17(4):495-499. https://doi.org/10.1038/nm.2324.
88. Schirmer L, Velmeshev D, Holmqvist S, et al. Neuronal vulnerability and multilineage diversity in multiple sclerosis. *Nature*. 2019;573(7772):75-82. https://doi.org/10.1038/s41586-019-1404-z.
89. Krasemann S, Madore C, Cialic R, et al. The TREM2-APOE pathway drives the transcriptional phenotype of dysfunctional microglia in neurodegenerative diseases. *Immunity*. 2017;47(3):566-581.e9. https://doi.org/10.1016/j.immuni.2017.08.008.
90. Masuda T, Sankowski R, Staszewski O, et al. Spatial and temporal heterogeneity of mouse and human microglia at single-cell resolution. *Nature*. 2019;566(7744):388-392. https://doi.org/10.1038/s41586-019-0924-x.
91. Liddelow SA, Guttenplan KA, Clarke LE, et al. Neurotoxic reactive astrocytes are induced by activated microglia. *Nature*. 2017;541(7638):481-487. https://doi.org/10.1038/nature21029.

92. Wheeler MA, Clark IC, Tjon EC, et al. MAFG-driven astrocytes promote CNS inflammation. *Nature*. 2020;578(7796):593-599. https://doi.org/10.1038/s41586-020-1999-0.
93. Hou B, Zhang Y, Liang P, et al. Inhibition of the NLRP3-inflammasome prevents cognitive deficits in experimental autoimmune encephalomyelitis mice via the alteration of astrocyte phenotype. *Cell Death Dis*. 2020;11(5):377. https://doi.org/10.1038/s41419-020-2565-2.
94. Molina-Gonzalez I, Miron VE. Astrocytes in myelination and remyelination. *Neurosci Lett*. 2019;713:134532. https://doi.org/10.1016/j.neulet.2019.134532.
95. Ray AK, DuBois JC, Gruber RC, et al. Loss of Gas6 and Axl signaling results in extensive axonal damage, motor deficits, prolonged neuroinflammation, and less remyelination following cuprizone exposure. *Glia*. 2017;65(12):2051-2069. https://doi.org/10.1002/glia.23214.
96. Gruber RC, Ray AK, Johndrow CT, et al. Targeted GAS6 delivery to the CNS protects axons from damage during experimental autoimmune encephalomyelitis. *J Neurosci*. 2014;34(49):16320-16335. https://doi.org/10.1523/JNEUROSCI.2449-14.2014.
97. Derfuss T, Parikh K, Velhin S, et al. Contactin-2/TAG-1-directed autoimmunity is identified in multiple sclerosis patients and mediates gray matter pathology in animals. *Proc Natl Acad Sci*. 2009;106(20):8302-8307. https://doi.org/10.1073/pnas.0901496106.
98. Biber K, Neumann H, Inoue K, Boddeke HWGM. Neuronal 'on' and 'off' signals control microglia. *Trends Neurosci*. 2007;30(11):596-602. https://doi.org/10.1016/j.tins.2007.08.007.
99. Thored P, Heldmann U, Gomes-Leal W, et al. Long-term accumulation of microglia with proneurogenic phenotype concomitant with persistent neurogenesis in adult subventricular zone after stroke. *Glia*. 2009;57(8):835-849. https://doi.org/10.1002/glia.20810.
100. Chang A, Smith MC, Yin X, Fox RJ, Staugaitis SM, Trapp BD. Neurogenesis in the chronic lesions of multiple sclerosis. *Brain*. 2008;131(9):2366-2375. https://doi.org/10.1093/brain/awn157.
101. Squarzoni P, Oller G, Hoeffel G, et al. Microglia modulate wiring of the embryonic forebrain. *Cell Rep*. 2014;8(5):1271-1279. https://doi.org/10.1016/j.celrep.2014.07.042.
102. Schirmer L, Merkler D, König FB, Brück W, Stadelmann C. Neuroaxonal regeneration is more pronounced in early multiple sclerosis than in traumatic brain injury lesions. *Brain Pathol*. 2013;23(1):2-12. https://doi.org/10.1111/j.1750-3639.2012.00608.x.
103. Cserép C, Pósfai B, Lénárt N, et al. Microglia monitor and protect neuronal function through specialized somatic purinergic junctions. *Science (80-)*. 2020;367(6477):528-537. https://doi.org/10.1126/science.aax6752.
104. Kato G, Inada H, Wake H, et al. Microglial contact prevents excess depolarization and rescues neurons from excitotoxicity. *eneuro*. 2016;3(3):ENEURO.0004-16.2016. https://doi.org/10.1523/ENEURO.0004-16.2016.
105. Masuda T, Amann L, Sankowski R, et al. Novel Hexb-based tools for studying microglia in the CNS. *Nat Immunol*. 2020;21(7):802-815. https://doi.org/10.1038/s41590-020-0707-4.
106. Kaiser T, Feng G. Tmem119-EGFP and Tmem119-CreERT2 transgenic mice for labeling and manipulating microglia. *eneuro*. 2019;6(4):ENEURO.0448-18.2019. https://doi.org/10.1523/ENEURO.0448-18.2019.
107. Pasciuto E, Burton OT, Roca CP, et al. Microglia require CD4 T cells to complete the fetal-to-adult transition. *Cell*. 2020;182(3):625-640.e24. https://doi.org/10.1016/j.cell.2020.06.026.
108. Böttcher C, Schlickeiser S, Sneeboer MAM, et al. Human microglia regional heterogeneity and phenotypes determined by multiplexed single-cell mass cytometry. *Nat Neurosci*. 2019;22(1):78-90. https://doi.org/10.1038/s41593-018-0290-2.
109. Li Q, Cheng Z, Zhou L, et al. Developmental heterogeneity of microglia and brain myeloid cells revealed by deep single-cell RNA sequencing. *Neuron*. 2019;101(2):207-223.e10. https://doi.org/10.1016/j.neuron.2018.12.006.
110. Jordão MJC, Sankowski R, Brendecke SM, et al. Single-cell profiling identifies myeloid cell subsets with distinct fates during neuroinflammation. *Science (80-)*. 2019;363(6425):eaat7554. https://doi.org/10.1126/science.aat7554.
111. Stoeckius M, Hafemeister C, Stephenson W, et al. Simultaneous epitope and transcriptome measurement in single cells. *Nat Methods*. 2017;14(9):865-868. https://doi.org/10.1038/nmeth.4380.
112. Chen W-T, Lu A, Craessaerts K, et al. Spatial Transcriptomics and in situ sequencing to study Alzheimer's disease. *Cell*. 2020;182(4):976-991.e19. https://doi.org/10.1016/j.cell.2020.06.038.
113. Geirsdottir L, David E, Keren-Shaul H, et al. Cross-species single-cell analysis reveals divergence of the primate microglia program. *Cell*. 2019;179(7):1609-1622.e16. https://doi.org/10.1016/j.cell.2019.11.010.
114. Galatro TF, Holtman IR, Lerario AM, et al. Transcriptomic analysis of purified human cortical microglia reveals age-associated changes. *Nat Neurosci*. 2017;20(8):1162-1171. https://doi.org/10.1038/nn.4597.
115. Muffat J, Li Y, Yuan B, et al. Efficient derivation of microglia-like cells from human pluripotent stem cells. *Nat Med*. 2016;22(11):1358-1367. https://doi.org/10.1038/nm.4189.
116. Pandya H, Shen MJ, Ichikawa DM, et al. Differentiation of human and murine induced pluripotent stem cells to microglia-like cells. *Nat Neurosci*. 2017;20(5):753-759. https://doi.org/10.1038/nn.4534.
117. Abud EM, Ramirez RN, Martinez ES, et al. iPSC-derived human microglia-like cells to study neurological diseases. *Neuron*. 2017;94(2):278-293.e9. https://doi.org/10.1016/j.neuron.2017.03.042.
118. Lin Y-T, Seo J, Gao F, et al. *APOE4* causes widespread molecular and cellular alterations associated with Alzheimer's disease phenotypes in human iPSC-derived brain cell types. *Neuron*. 2018;98(6):1141-1154.e7. https://doi.org/10.1016/j.neuron.2018.05.008.
119. Hasselmann J, Coburn MA, England W, et al. Development of a chimeric model to study and manipulate human microglia in vivo. *Neuron*. 2019;103(6):1016-1033.e10. https://doi.org/10.1016/j.neuron.2019.07.002.
120. Svoboda DS, Barrasa MI, Shu J, et al. Human iPSC-derived microglia assume a primary microglia-like state after transplantation into the neonatal mouse brain. *Proc Natl Acad Sci*. 2019;116(50):25293-25303. https://doi.org/10.1073/pnas.1913541116.
121. Xu R, Li X, Boreland AJ, et al. Human iPSC-derived mature microglia retain their identity and functionally integrate in the chimeric mouse brain. *Nat Commun*. 2020;11(1):1577. https://doi.org/10.1038/s41467-020-15411-9.
122. Bennett FC, Bennett ML, Yaqoob F, et al. A combination of ontogeny and CNS environment establishes microglial identity. *Neuron*. 2018;98(6):1170-1183.e8. https://doi.org/10.1016/j.neuron.2018.05.014.
123. Cronk JC, Filiano AJ, Louveau A, et al. Peripherally derived macrophages can engraft the brain independent of irradiation and maintain an identity distinct from microglia. *J Exp Med*. 2018;215(6):1627-1647. https://doi.org/10.1084/jem.20180247.
124. Mertens J, Paquola ACM, Ku M, et al. Directly reprogrammed human neurons retain aging-associated transcriptomic signatures and reveal age-related nucleocytoplasmic defects. *Cell Stem Cell*. 2015;17(6):705-718. https://doi.org/10.1016/j.stem.2015.09.001.
125. Mazaheri F, Snaidero N, Kleinberger G, et al. TREM2 deficiency impairs chemotaxis and microglial responses to neuronal injury. *EMBO Rep*. 2017;18(7):1186-1198. https://doi.org/10.15252/embr.201743922.

126. Willis EF, MacDonald KPA, Nguyen QH, et al. Repopulating microglia promote brain repair in an IL-6-dependent manner. *Cell*. 2020;180(5):833-846.e16. https://doi.org/10.1016/j.cell.2020.02.013.
127. Moroni F, Dwyer BJ, Graham C, et al. Safety profile of autologous macrophage therapy for liver cirrhosis. *Nat Med*. 2019;25(10):1560-1565. https://doi.org/10.1038/s41591-019-0599-8.

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